Science in China Series C: Life Sciences

Springer

www.scichina.com
life.scichina.com
www.springer.com/scp
www.springerlink.com

Article

Water filtration rate and infiltration/accumulation of low density lipoproteins in 3 different modes of endothelial/smooth muscle cell co-cultures

DING ZuFeng, FAN YuBo & DENG XiaoYan†

School of Biological Science & Medical Engineering, Beihang University, Beijing 100191, China

Using different endothelial/smooth muscle cell co-culture modes to simulate the intimal structure of blood vessels, the water filtration rate and the infiltration/accumulation of LDL of the cultured cell layers were studied. The three cell culture modes of the study were: (i) The endothelial cell monolayer (EC/Φ); (ii) endothelial cells directly co-cultured on the smooth muscle cell monolayer (EC-SMC); (iii) endothelial cells and smooth muscle cells cultured on different sides of a Millicell-CM membrane (EC/SMC). It was found that under the same condition, the water filtration rate was the lowest for the EC/SMC mode and the highest for the EC/Φ mode, while the infiltration/accumulation of DiI-LDLs was the lowest in the EC/Φ mode and the highest in the EC-SMC mode. It was also found that DiI-LDL infiltration/accumulation in the cultured cell layers increased with the increasing water filtration rate. The results from the *in vitro* model study therefore suggest that the infiltration/accumulation of the lipids within the arterial wall is positively correlated with concentration polarization of atherogenic lipids, and the integrity of the endothelium plays an important role in the penetration and accumulation of atherogenic lipids in blood vessel walls.

cell culture mode, atherosclerosis, concentration polarization

In the past years, many researchers have emphasized the importance of wall shear stress in atherogenesis. However, due to the fact that the early event leading to the genesis of atherosclerosis is the accumulation of cholesterol and other lipids within the arterial wall, in recent years, researchers have been paying more and more attention to material transport in the circulation and the interactions between blood cells and the blood vessel walls[1], and proposed the concepts of "residence time" for atherogenic agents[2,3]. They believe that the "residence time" and the deposition of atherogenic particles onto the blood vessel walls[4] play important roles in atherogenesis and thrombus formation.

Deng et al.[5] has predicted a mass transport phenomenon of concentration polarization of atherogenic low density lipoproteins (LDL) within the arterial system theoretically and verified it experimentally *in vitro*[6,7]. Their study revealed that concentration polarization of LDL was positively correlated with water filtration rate across the arterial wall and adversely correlated with flow-induced wall shear stress. Apparently, the occurrence of concentration polarization of LDL in the arterial system can affect the "residence time" and the deposition of atherogenic particles. They therefore have suggested that flow-dependent LDL concentration polarization at the blood/wall interface may play an important role in the localization of atherogenesis.

Now that concentration polarization of LDL at the blood/wall interface has a positive correlation with water filtration rate and affects the infiltration/accumulation of LDLs within the arterial wall, which is the early event leading to atherogenesis, our questions are: What roles

Received January 29, 2009; May 18, 2009
doi: 10.1007/s11427-009-0135-z
†Corresponding author (email: dengxy1953@buaa.edu.cn)
Supported by Grants-in-Aid from the National Natural Science Foundation of China (Grant Nos. 10632010 and 30670517) and the Innovation Foundation of BUAA for PhD Graduates.

Citation: DING Z F, FAN Y B, DENG X Y. Water filtration rate and infiltration/accumulation of low density lipoproteins in 3 different modes of endothelial/smooth muscle cell co-cultures. Sci China Ser C-Life Sci, 2009, 52(11): 1023-1029, doi: 10.1007/s11427-009-0135-z

do the integrity of the endothelial surface and the structure of the blood vessel wall play in atherogenesis? How do they affect water filtration rate of the blood vessel and the infiltration/accumulation of atherogenic lipids in the vessel wall? In order to answer these questions, in the present study, we used 3 different modes of cell cultures to simulate the blood vessel walls with different morphological states and studied the infiltration and accumulation of LDL in the cultured cell layers.

1 Materials and methods

1.1 Materials

RPMI 1640 medium was purchased from Invitrogen Corpration (Calif, USA). Dulbecco's Modified Eagle Medium (DMEM), penicillin and streptomycin were from Sigma Chemical (Stlouis, MO). Fetal calf serum (FCS) was from TBD (Beijing, China). Millicell-CM culture inserts (PICM 03050) were from Millipore Corp. (Bedford, MA). 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine (DiI) was from Biomedical Technoligies Inc. (MA, USA).

1.2 Preparation of LDL and DiI-LDL

LDLs were prepared and purified from fresh plasma obtained from healthy volunteers by gradient ultracentrifugation according to the method of Redgrave et al.[8]. The purity of the LDLs prepared was over 90% (LDL/total proteins), which was determined by the method of Lowry et al.[9]. The LDLs were stored in sterile polystyrene tubes for periods less than 5 d at 4℃ until ready for use, therefore the level of oxidation of the LDLs was very low. Prior to each perfusion experiment, the purified LDLs were labeled with DiI following the procedure described by Li et al.[10]. The labeled LDLs were added to the DMEM cell culture medium that was used as the experimental perfusion solution. Then, the concentration of the labeled LDLs in the prepared perfusion solution was determined by measuring the protein concentration of the labeled LDLs by Bradford Protein Assay Kit.

1.3 Primary human endothelial and smooth muscle cells

Human ECs (huECs) and SMCs (huSMCs) were isolated from human umbilical cords. First, blood in the umbilical cord was rinsed with cold phosphate-buffered saline (PBS) buffer. After 20 mL of 0.125% trypsinase was injected into the vein of the cord, the cord was incubated at (37±1)℃ for 20 min. Then, huECs were collected by perfusion with the 1640 cell culture medium. Next, the collected ECs were centrifuged for 5 min at 89×*g* and suspended in 1640 medium containing 20% fetal calf serum (FCS). HuSMCs were acquired by explanting pieces of tissue individually. HuECs and huSMCs were cultured separately in RPMI 1640 Medium and DMEM with FCS (20%, v/v), penicillin (100 kU/L) and streptomycin (100 mg/L) in a humidified environment of 5% CO_2 and 95% air at (37±1)℃.

1.4 HuEC and HuSMC co-culture preparations

The 3 cell cultures with different modes were prepared as follows: EC/Φ mode (endothelial cell monolayer culture): Human endothelial cells (huECs) at a density of 1×10^6 cells/cm^2 were seeded on a Millicell-CM membrane (PICM 03050; Millipore Corp., Bedford, MA) which had an effective surface area of 4.2 cm^2 with pores of 0.4 μm in diameter and was incubated for about 3 h at (37±1)℃. Then, the cell culture was immersed in a culture medium. The state of attachment of cells onto the membrane and confluence of cells on the membranes were monitored by a phase contrast microscope from time to time. The culture medium was renewed every 3—4 days.

EC-SMC mode (direct endothelial cell and smooth muscle cell co-culture): Human smooth muscle cells (HuSMCs) at a density of 1×10^6 cells/cm^2 were seeded on a Millicell-CM membrane for 3—4 days at (37±1)℃. After the confirmation by phase-contrast microscopy that the entire surface of the membrane was covered with huSMCs, huECs were seeded directly over the huSMCs at a density of 1×10^6 cells/cm^2. In order to assure the huECs to form a confluent monolayer overlaying on top of the whole surface of the cultured huSMCs, the co-cultured cells were incubated at (37±1)℃ for 2 d before the experiment.

EC/SMC mode (indirect endothelial cell and smooth muscle cell co-culture): First, huECs at a density of 1×10^6 cells/cm^2 were seeded on the basal side of a Millicell-CM membrane. Two hours after, the membrane with huECs was placed into a 6-well plate containing the cell culture medium. Then, the inner side of the membrane was seeded with huSMCs. Following the same procedure of cell culture as the EC-SMC mode, the ECs and SMCs separated by the semi-permeable

Millicell- CM membrane were co-cultured.

1.5 Experimental setup

Figure 1 shows a schematic drawing of the experimental perfusion system. It consisted of a head tank, a downstream collecting reservoir, a modified parallel-plate flow chamber with a height of 0.5×10^{-3} m and a width of 35×10^{-3} m, a peristaltic flow pump to circulate the perfusion fluid (the cell culture medium) and a blender of air and CO_2 with a constant temperature ((37±1)℃). All the components of the perfusion system were connected using tygon tubing. The flow rate was controlled by adjusting the height of the overflow head tank and the resistance of the needle valve so that both the desired flow rate and a perfusion pressure could be achieved simultaneously. As an insert, the prepared Millicell-CM membrane dish with cell cultures of different modes (the cell culture insert) was installed at the middle bottom of the flow chamber with the help of a specially designed support. The EC side of the cell culture insert was exposed to the flow. The abluminal side of the cell culture insert was supported with a hand-made membrane support so that the co-culture did not sag even when perfusion pressure was applied to it. A pressure transducer and a flow meter were used to monitor the perfusion pressure and the flow rate through the flow chamber, respectively. During the experiment, the flow chamber was enclosed in a container to keep it at a constant temperature of (37±1)℃.

1.6 Perfusion solution

For each experiment, cell culture medium DMEM with 10% fetal calf serum was freshly prepared as the experimental perfusion solution, in which DiI-LDL was added at a desired concentration. The pH value of the perfusion solution was adjusted to 7.2.

1.7 Measurement of water filtration rates

Water filtration rates (v_w) across the cell culture preparations of different modes were measured following the same procedure described by Deng et al[11]. with the help of the calibrated pipette on the dish support, which had an inner diameter of 1×10^{-3} m (Figure 1). During the measurement, wall shear stress and DiI-LDL concentration of the perfusion solution were kept constant at 1.3 Pa and 10 μg/mL, respectively, while the perfusion pressure within the flow chamber was varied at 10, 30, 50, 70 and 100 mmHg.

Wall shear stress was calculated using the formula as follows: $\tau = 6\,\mu Q/wh^2$, where μ is the viscosity of the medium and Q is the flow rate (milliliters per second), h and w are the width and height of the parallel-plate flow chamber.

1.8 Measurement of DiI-LDL infiltration/accumulation

In order to evaluate the role of the integrity of the endothelium and the structure of the cell culture layers in DiI-LDL infiltration/accumulation that is greatly affect-

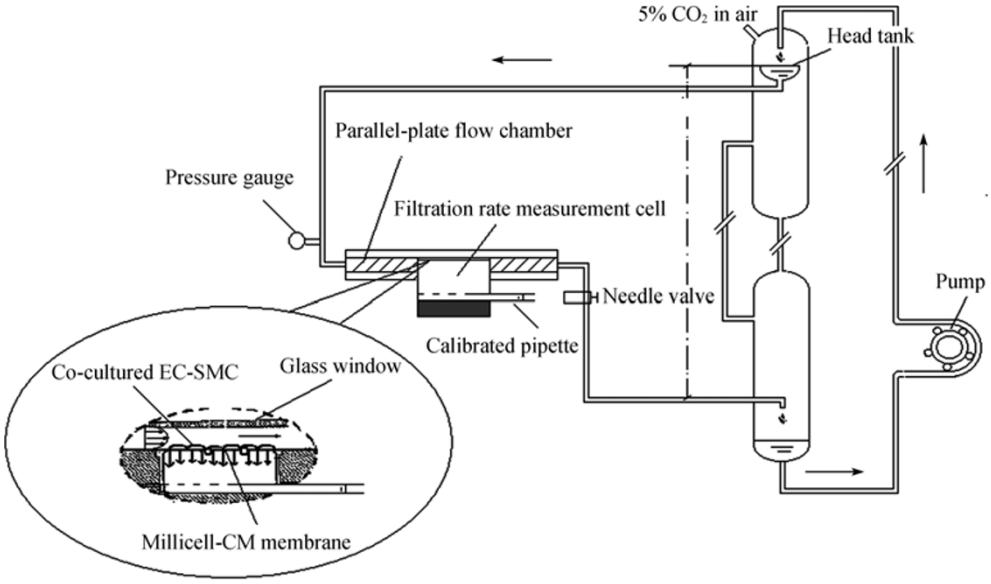

Figure 1 Schematic drawing of the experiment perfusion system. The cell culture insert was the main component of the experiment perfusion system. The overflow head-tank provided a steady flow to the parallel-plate flow chamber. The filtration rate measurement cell on the abluminal side of the co-culture was filled with the same fluid as the perfusate.

ed by the surface concentration (c_w) of DiI-LDLs, c_w of DiI-LDLs has to be kept constant in all experiments. According to the study by Deng et al.[5,6], c_w is a function of the bulk concentration (c_0) of DiI-LDLs, the water filtration rate (v_w) and the wall shear stress (τ_w). Therefore, the best way to maintain c_w constant in all experiments is to set the 3 parameters (c_0, v_w and τ_w) constant.

The measurement of DiI-LDL infiltration/accumulation was carried out under 2 groups of conditions: (i) Setting c_0, τ_w and v_w at 10 μg/mL, 1.3 Pa and 4.0×10^{-6} cm/s; (ii) setting c_0, τ_w and v_w at 10 μg/mL, 1.3 Pa and 5.3×10^{-5} cm/s. DiI-LDL infiltration/accumulation within the cell-culture preparations was determined using a slightly modified method described by others[12]. Briefly, after 2 h perfusion flow, the cell culture insert was disassembled from the flow chamber and washed with cold PBS for 3 times. These cells were lysed with 1 mL of 0.4% Triton-X in PBS for 10 min and then detached from the membrane using a cell scraper. Complete lysis of the cells was achieved by gentle pipetting of the lysate, followed by removal of cell debris by centrifugation. The fluorescence intensity of the samples was measured with a spectrofluorometer (Cary Eclipse, Varian, USA) at excitation and emission wavelengths of 514 and 550 nm, respectively. The fluorescence intensity was then converted to the concentration of lipoproteins with a calibration curve of DiI-LDL. A standard procedure was followed throughout the entire experiment. Prior to the exposure of the co-cultured cell insert to DiI-LDL, the cell insert was first subjected to steady laminar shear flow of 1.2 Pa for 24 h to precondition the ECs. Prior to the completion of the measurement, the water filtration rate across the cell-culture preparation was measured again to assure it was unchanged.

1.9 Statistical analysis

Experiments were repeated at least 3 times under the same flow conditions. Statistical analyses were performed by using the Origin 7.0 software. The results were presented as means ± SD. Differences in means were considered significant if $P < 0.05$.

3 Results

3.1 The ECs and SMCs primary culture from human umbilical cords

Figure 2 shows that the ECs and SMCs primary culture from human umbilical cords with the methods of trypsinization and explanting pieces of tissue individually, respectively. The ECs were incubated for 3 days after isolation from human umbilical cords in 1640 culture medium, while the SMCs were incubated for 10 d in DMEM at (37 ±1)℃. Under the static condition, the shape of ECs cultured alone was round or polygonal. It was typical contact-inhibited cobblestone appearance. Differently, the shape of SMCs cultured alone was overlapping layers with a typical hill-and-valley pattern of growth. In the co-cultures of ECs and SMCs, owing to interference by the porous membrane and underlying co-cultured SMCs, the growth pattern of the ECs in the co-cultures was not clearly observable under the microscope.

3.2 Correlation of water filtration rate with perfusion pressure

In this set of experiment, the perfusion pressure within the flow chamber was varied at 10, 30, 50, 70 and 100 mmHg while keeping the shear stress and the DiI-LDL concentration of the perfusion solution constant at 1.3 Pa

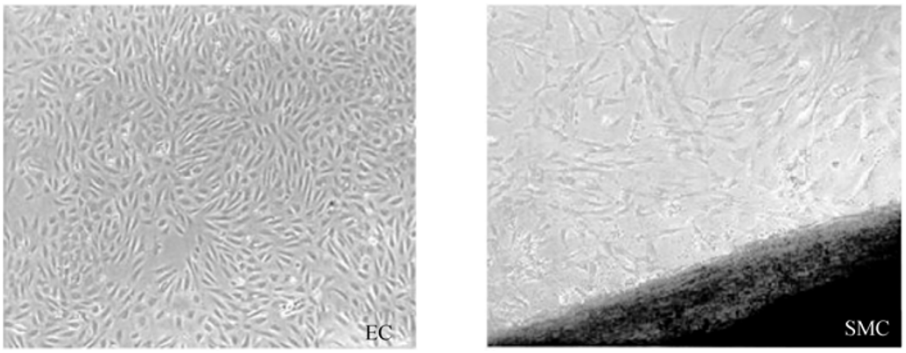

Figure 2 Photographs of human ECs and SMCs primary culture. The cells were incubated for 3 and 10 days in a culture medium at (37±1)℃ for ECs and SMCs, respectively. The opaque part in SMC was explanting pieces of the tissue from human umbilical cords.

and 10 μg/mL. The measured results are shown in Figure 3. A regression analysis was performed for data fitting. As evident from the figure, the water filtration rate (v_w) across the semi-permeable wall of the cell culture insert increased almost linearly with increasing perfusion pressure for all of the 3 modes of cell cultures. The measured water filtration rates were ranged from 2.0×10^{-6} to 6.5×10^{-5} cm/s. The measurement showed that under the same perfusion pressure, v_w was the highest for EC/Φ and the lowest for EC/SMC.

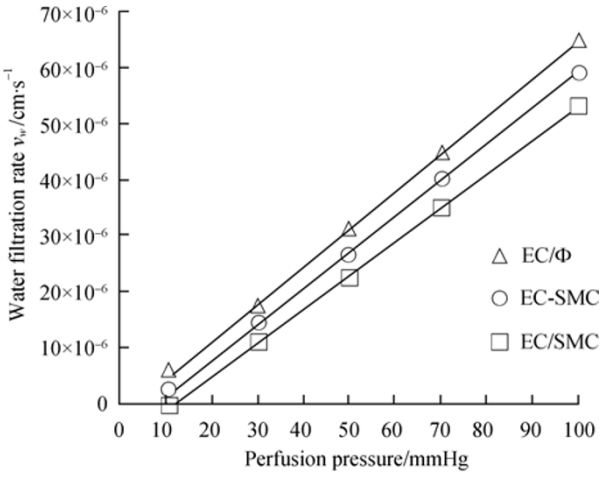

Figure 3 The plots of the filtration rate vs. perfusion pressure for the 3 different modes of cell cultures. The shear stress and the concentration of DiI-LDL were kept constant during the measurement at 1.3 Pa and 10 μg/mL, respectively. The results are expressed as means±SD (n= 8).

3.3 DiI-LDL infiltration/accumulation

In this set of experiment, 2 groups of conditions were set. c_0, τ_w and v_w were: (i) 10 μg/mL, 1.3 Pa and 4.0×10^{-6} cm/s, respectively and (ii) 10 μg/mL, 1.3 Pa and 5.3×10^{-5} cm/s, respectively. From Figure 3, it can be determined that if v_w is set at 4.0×10^{-6} cm/s, the corresponding perfusion pressures for the 3 modes of cell cultures must be 10, 14 and 19 mmHg for EC/Φ, EC-SMC and EC/SMC, respectively. The same as above, when v_w is set at 5.3×10^{-5} cm/s, the corresponding perfusion pressures must be 83, 90 and 100 mmHg for EC/Φ, EC-SMC and EC/SMC, respectively. During the measurement, the determined perfusion pressures were used for their corresponding modes of cell-culture preparations.

Figure 4 shows the values of DiI-LDL infiltration/accumulation for EC/Φ, EC/SMC and EC-SMC while the water filtration rate was set at 4.0×10^{-6} cm/s and 5.3×10^{-5} cm/s respectively. As evident from the figure, the infiltration/accumulation of DiI-LDL was the lowest for the EC/Φ mode and the highest for the EC-SMC mode. The infiltration/accumulation of DiI-LDL increased very sharply when v_w increased from 4.0×10^{-6} cm/s to 5.3×10^{-5} cm/s. At v_w= 4.0×10^{-6} cm/s, it was (0.12 ± 0.03) μg/h for EC/Φ, 0.16 ± 0.05 μg/h for EC-SMC and (0.18 ± 0.08) μg/h for EC/SMC. When v_w increased to 5.3×10^{-5} cm/s, the value of DiI-LDL infiltration/accumulation increased to (0.44 ± 0.02), (0.56 ± 0.04) and (0.62 ± 0.05) μg/h for EC/Φ, EC-SMC and EC/SMC, respectively.

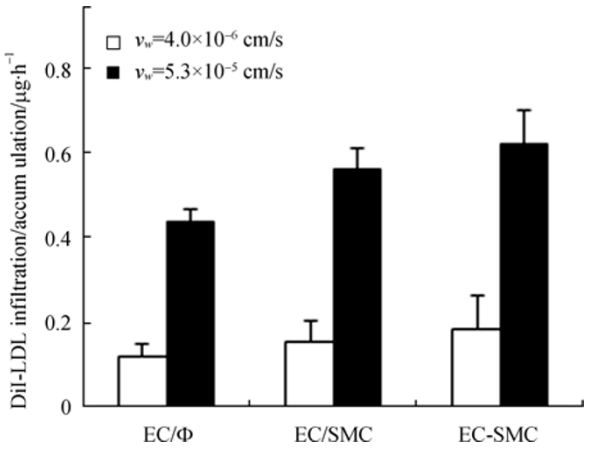

Figure 4 DiI-LDL infiltration/accumulation for the 3 different modes of cell cultures. The shear stress and the concentration of DiI-LDL were kept constant during the measurement at 1.3 Pa and 10 μg/mL, respectively. The results are expressed as a means+SD (n=3~5).

4 Discussion

Atherogenic lipid infiltration and accumulation within a blood vessel wall may depend not only on the concentration of lipids at the blood/arterial wall interface, but also on the integrity of the endothelial surface and the structure of the blood vessel wall. In order to verify this, we used three modes of cell cultures to simulate the walls of blood vessels with different morphological states, and measured the water filtration rate and DiI-LDL infiltration/accumulation in the cultured cell preparations.

The three cell culture modes of the study were: (i) The endothelial cell monolayer (EC/Φ); (ii) endothelial cells directly co-cultured on the smooth muscle cell monolayer (EC-SMC)[12]; (iii) endothelial cells and smooth muscle cells cultured on different sides of a

Millicell-CM membrane (EC/SMC)[13]. Morphological observation revealed that, similar to the EC/Φ mode, the confluent EC layer of the EC/SMC mode was tight and intact, while its co-cultured SMCs were uniformly distributed in the absence of the hill-and-valley pattern, which was consistent with what observed by Powell et al.[14]. Therefore, during the flow experiment, the co-cultured SMCs did not directly subject to the flow. Differently, the endothelial cells of the EC-SMC mode did not 100% cover the surface of the co-cultured SMCs underneath, leaving some of the SMCs directly exposed to the perfusion flow. Therefore, the co-culture preparations of the EC-SMC mode can be used to analogy blood vessels with endothelial damage.

In the present study, the concentration of DiI-LDLs at the luminal surface of the cultured cell preparations (c_w) was kept constant in all experiments by maintaining the water filtration rate, wall shear rate and the bulk concentration (c_0) of DiI-LDLs constant. The experiment showed that, under the same condition (the same perfusion pressure and the same wall shear stress), the water filtration rate across the cultured cell preparations was the lowest for the EC/SMC mode and the highest for the EC/Φ mode, while the infiltration/accumulation of DiI-LDLs was the lowest for the EC/Φ mode and the highest for the EC-SMC mode. The results also demonstrated that the amount of DiI-LDL infiltration/accumulation increased with the increasing water filtration rate. When v_w increased from 4.0×10^{-6} to 5.3×10^{-5} cm/s, the DiI-LDL infiltration/accumulation in all three modes of cell cultures increased very sharply. According to the study by Deng et al.[5], the luminal surface concentration of DiI-LDLs (c_w) was almost linearly correlated to v_w, we therefore can conclude that the DiI-LDL infiltration/accumulation by the cultured cell preparations was determined by both the c_w and the modes of the cell cultures.

The experimental result revealed that, under the same perfusion pressure, although the EC/Φ mode (ECs cultured alone) had the highest water filtration rate, the DiI-LDL infiltration/accumulation of this mode was the lowest. This result may not necessarily imply that the amount of DiI-LDL infiltration into the EC/Φ preparation was the lowest, but most likely indicating that most of the DiI-LDLs infiltrating into the culture cell preparation passed through the preparation and effluxed from its opposite side. The infiltration/accumulation of DiI-LDLs observed for the EC/Φ preparation can be used to analogy the situation of the vein. As we all know, atherosclerosis only develops in arteries, not in veins. The reason for this can hardly be explained only by the shear stress hypothesis proposed by many researchers[15,16], because the veins also branch and experience flow disturbances with low wall shear stresses. However, from the view point of mass transport, this phenomenon can be easily explained. As it has been known that an early event leading to the genesis and development of atherosclerosis is the accumulation of cholesterol and other lipids within the blood wall. However, greater accumulation of atherogenic lipids within a blood vessel wall is supposed to be due to increased influx of lipids into the wall and decreased efflux of the lipids out of the wall. In other words, within a blood vessel wall, the final net lipid accumulation, leading to atherosclerotic lesions, is the result of 2 processes: influx and efflux of lipids. In the circulation, the veins may have the same level of lipid influx as the arteries because both the veins and the arteries have the same level of water filtration rate under their own physiological pressure condition[17], hence exposing to the same level of luminal surface lipid concentration[5]. However, due to the fact that the veins have much thinner walls than the arteries, therefore, just like the EC/Φ mode cell culture preparation, the efflux of the lipids infiltrating into the venous walls may be much higher than that of the arterial wall. This leads to the net accumulation of atherogenic lipids within the venous walls much lower than that within the arterial walls. This might be the main reason why atherosclerosis only develops in the arterial tree, not in the venous system.

However, when veins are implanted into the arterial system as grafts or subjected to arterial pressure as in the case of arteriovenous fistulas, accelerated atherogenisis would occur. The arteriographic examinations by Campeau et al.[18] showed that 1 year after implantation, 32% of the venous grafts developed significant atherosclerotic stenosis and that another 30% were occluded. The accelerated atherosclerosis to venous grafts is though largely related to the arterialization process of the venous grafts, a remodeling adaptive reaction to arterial pressure[19], but the mechanism of lipids transport observed in this study may play a very important role in it.

In the present experimental study, among the 3 cell culture modes, the endothelial cell morphology of the EC-SMC mode is most similar to the intima with endothelial lining damage. The results showed that the cul-

ture cell preparation of EC-SMC mode had the highest lipid infiltration/accumulation and the second highest water filtration rate. Therefore, the intima of a venous graft bears an analogy to the direct co-culture mode (EC-SMC). When a vein is harvested and prepared for arterial reconstructive surgery, the endothelial surface of the vein will no doubt be damaged more or less. After implantation, when subjected to arterial pressure, the venous graft will experience excessive distension, which can further damage the endothelial linings of the vessel and result in an increased water filtration flow[20]. Therefore, similar to the culture cell preparation of the EC-SMC mode, the arterialized venous graft with its thickened wall and damaged endothelial linings will have a much higher rate of lipid infiltration/accumulation within its wall, hence leading to the accelerated atherogenesis.

Previous studies by Berceli et al.[21] showed that the LDL permeability into the arterial wall was high in low shear stress regions where the endothelial cells were round and polygonal with no preferred orientation, while the LDL permeability was low in high shear stress regions where the endothelial cells were enlongated and aligned to the direction of blood flow. Therefore, the infiltration/accumulation within the culture cell preparations of the EC-SMC mode may also be used to analogize the intima of the arterial walls in disturbed flow regions with low wall shear stresses.

In summary, the present experimental study demonstrated that the mass transport phenomenon of atherogenic lipid concentration polarization[5], the integrity of the endothelial surface and the structure of the blood vessel wall may play very important roles in atherogenesis and can be used to not only account for the localization of atherosclerosis, but also explain why atherogenesis only occurs in the arterial tree, not in the venous system. Based on this explanation, we believe that a rigid external wrap support to the venous graft may prevent or slow down the atherogenic process of venous grafts by reducing the damage to the endothelium and the infiltration/accumulation of lipids within the venous wall.

1 Kleinstreuer C, Hyun S, Buchanan J R. Hemodynamic parameters and early intimal thickening in branching blood vessels. Crit Rev Biomed Eng, 2001, 29: 1—64
2 Ku D N, Giddens D P. Pulsatile flow in a model carotid bifurcation. Arteriosclerosis, 1983, 3: 31—39
3 Buchanan J R, Kleinstreuer C, Comer J K. Rheologica effects on pulsatile hemodynamics in a stenosed tube. Comput Fluids, 2000, 29: 695—724
4 Hyun S, Kleinstreuer C, Archie J P. Computational particle- hemodynamics analysis and geometric reconstruction after carotid endarterectomy. Comput Biol Med, 2001, 31: 365—384
5 Deng X Y, Marois Y, How T, et al. Luminal surface concentration of lipoprotein (LDL) and its effect on the wall uptake of cholesterol by canine carotid arteries. J Vasc Surg, 1995, 21: 135—145
6 Wang G X, Deng X Y, Guidoin R. Concentration polarization of macromolecules in canine carotid arteries and its implication for the localization of atherogenesis. J Biomech, 2004, 36: 45—51
7 Zhang Z G, Deng X Y, Fan Y B, et al. *Ex vitro* experimental study on concentration polarization of macromolecules (LDL) at an arterial stenosis. Science in China Series C-Life Sci, 2007, 50: 486—491
8 Redgrave T G, Roberts D C K, West C E. Separation of plasma lipoproteins by density-gradient ultracentrifugation. Anal Biochem, 1974, 65: 42—49
9 Lowry O H, Rosebrough N J, Farr A L, et al. Protein measurement with the folin phenol reagent. J Biol Chem, 1951, 193: 267—275
10 Li H, Zhang Z H, Blessington D. Carbocyanine labeled LDL for optical imaging of tumors. Acad radiol, 2004, 11: 669—677
11 Deng X Y, Marois Y, Guidoin R. Fluid filtration across the arterial wall under flow conditions: Is wall shear rate another factor affecting filtration rate? Ann NY Acad Sci, 1998, 858: 105—115
12 Niwa K, Karino T, Sakai J. The effects of a shear flow on the uptake of LDL and acetylated LDL by an EC monoculture and an EC-SMC coculture. Ann Biomed Eng, 2004, 32: 537—543
13 Chiu J J, Chen L J, Chen C N, et al. A model for studying the effect of shear stress on interactions between vascular endothelial cells and smooth muscle cells. J Biomech, 2004, 37: 531—539
14 Powell R J, Bhargava J, Basson M D, et al. Coculture conditions alter endothelial modulation of TGF-β1 activation and smooth muscle growth morphology. Am J Physiol, 1998, 274: H642—649
15 Caro C G. Vascular fluid dynamics and vascular biology and disease. Math Meth Appl Sci, 2001, 24: 1311—1324
16 Malek A M, Alper S L, Lzumo S. Hemodynamic shear stress and its role in atherosclerosis. JAMA, 1999, 282: 2035—2042
17 Wilens S L, McCluskey R T. The comparative filtration properties of excised arteries and veins. Am J Med Sci, 1952, 224: 540—547
18 Campeau L C, Enjalbert M, Lesperance J, et al. The relation of risk factors to the development of atherosclerosis in saphenous-vein bypass grafts and progression of disease in native circulation. New Engl J Med, 1984, 311: 1329—1332
19 Walton K W, Slaney G, Ashton F. Atherosclerosis in vascular grafts for peripheral vascular disease. I. Autogenous vein grafts. Atherosclerosis, 1985, 54: 49—64
20 Berceli S A, Brorovetz H S, Sheppeck R A, et al. Mechanisms of vein graft atherosclerosis: LDL metabolism and endothelial actin reorganization. J Vasc Surg, 1991, 13: 336—347
21 Berceli S A, Warty V S, Sheppeck R A, et al. Hemodynamics and low density lipoprotein metabolism: Rates of low density lipoprotein incorporation an degradation along medial and lateral walls of the rabbit aorto-iliac bifurcation. Arteriosclerosis, 1990, 10: 686—694